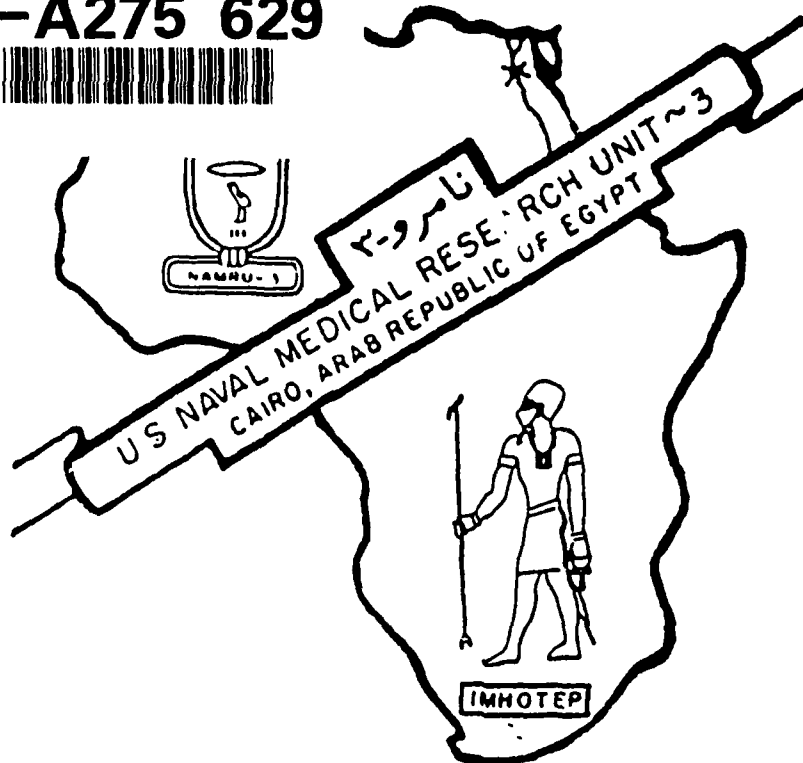AD-A275 629

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VERTICAL TRANSMISSION OF WEST NILE VIRUS BY <u>CULEX</u>
AND <u>AEDES</u> SPECIES MOSQUITOES

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VERTICAL TRANSMISSION OF WEST NILE VIRUS BY *CULEX* AND *AEDES* SPECIES MOSQUITOES

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Abstract. Experiments were conducted to determine whether West Nile (WN) virus was transmitted vertically by colonized strains of *Aedes albopictus*, *Ae. aegypti*, and *Culex tritaeniorhynchus*. Female mosquitoes were infected by intrathoracic inoculation with WN virus, and the F_1 progeny were tested for virus by the fluorescence antibody technique and the newborn mouse assay. Each of the three mosquito species transmitted WN virus to F_1 adults derived from immature forms reared at 26°C. The minimal filial infection rate (MFIR) ranged from 1:124 to 1:138 for *Ae. albopictus*, from 1:62 to 1:172 for *Ae. aegypti*, and from 1:325 to 1:859 for *Cx. tritaeniorhynchus*. The MFIR for *Cx. tritaeniorhynchus* reared at 20°C was 1:213 for larvae and 1:390 for pupae, and 1:208 for larvae and 1:554 for pupae reared at 26°C. These data are the first reported evidence of vertical transmission of WN virus by mosquitoes, and therefore warrant further studies to determine whether vertical transmission occurs among WN viral-infected mosquitoes in nature.

West Nile (WN) virus is an important human pathogen that has caused large epidemics of febrile disease in Africa, Asia, and Europe.[1] These epidemics have coincided with an increase in the population density of mosquitoes, especially *Culex* species, during the summer in temperate regions and during the rainy season in the tropics. West Nile virus has been isolated from several species of *Culex*, and to a lesser extent from *Aedes* and *Anopheles* mosquitoes, and argasid and ixodid ticks.[1] However, most isolates have been obtained from *Cx. univittatus* in Egypt,[2] Israel,[3] and the Republic of South Africa,[4, 5] *Cx. molestus* in France,[6] and the *Cx. vishnui* complex, including *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, and *Cx. vishnui* in India[7] and Pakistan.[8] Experimental studies have shown that several of these *Culex* species were readily infected with WN virus and capable of transmitting the virus to laboratory animals.[9-13] Evidence of WN viral infection has been demonstrated in several species of domestic and wild vertebrates, but only wild birds have been incriminated as viral-amplifying hosts.[14-20] These ecologic and epidemiologic observations indicated that the primary maintenance and transmission cycle of WN virus during the summer season involved *Culex* species mosquitoes and wild birds.

The maintenance mechanism(s) for WN virus during periods unfavorable for adult mosquito activity, especially during the winter in temperate regions is unknown. Initial experimental attempts to demonstrate vertical transmission of the virus by *Cx. tritaeniorhynchus* and *Ae. aegypti* as a possible overwintering mechanism were unsuccessful.[13, 21] Since vertical transmission by mosquitoes of other flaviviruses has been conclusively demonstrated,[22-27] this study was conducted to retest the hypothesis that WN virus could be transmitted vertically by *Culex* and *Aedes* species mosquitoes.

MATERIALS AND METHODS

Mosquitoes

Culex tritaeniorhynchus, *Ae. albopictus*, and *Ae. aegypti* used in these experiments during 1979 and 1980 were obtained from colonies established during 1968 and 1969 from adults and immature forms collected in Pakistan. Immature mosquitoes were reared at 26°C for sustaining colonies, and at 20°C and 26°C for use in exper-

iments. All adults were maintained at 28°C as described previously.[11]

Virus

The Egypt 101 strain of WN virus, originally isolated in 1951 from human serum in Egypt, was used in all experiments.[28] The virus stock used in the first series of experiments had received 16 intracerebral passages in newborn mice, and four intrathoracic passages in Cx. tritaeniorhynchus. Suspensions prepared from parent mosquitoes that were infected in the first experiments were used as the source of virus for all subsequent experiments. Stock virus was stored in aliquots at −70°C until used to infect mosquitoes.

Infection of mosquitoes

Caged-mated female mosquitoes were inoculated intrathoracically with 1.0 µl of a 1:10 dilution of WN virus 3–5 days after emergence. The virus was diluted in 0.75% bovine phosphate albumin (BPA). On the second and third days after inoculating mosquitoes, adult mice were provided as a source of blood to induce egg production. Engorged mosquitoes were transferred to separate cages and held individually, or in batches depending on the objective of the experiment. West Nile virus infection of the parental female mosquitoes in all experiments was confirmed by methods described below.

The initial experiments were designed to consider the possibility that WN virus was transmitted vertically by mosquitoes. Eggs that were laid by groups of separate species of infected mosquitoes were hatched and the immature forms were reared at 28°C as described previously.[13] Newly emerged F₁ adults were kept separately according to species and sex as pools of 10–45 at −70°C until assayed for virus.

A second experiment was conducted to determine the effects of temperature on WN viral infection rates among immature progeny of infected Cx. tritaeniorhynchus. Eggs were hatched and equal numbers of the first-stage larvae were reared at 20°C or 28°C. The third- and fourth-stage larvae or the pupae were stored as separate pools of 75–100 larvae each at −70°C until assayed for virus.

Virus assays

Suspensions of parental and of F₁ mosquitoes were prepared for virus assay by homogenizing individual or pooled specimens in sterile mortars with 1.0 ml of BPA supplemented with 200 units/ml of penicillin and 200 µg/ml of streptomycin. The suspensions were clarified by centrifugation at 12,000 × g for 20 min at 4°C. Each undiluted suspension was tested for virus by inoculating 1.0 µl intrathoracically into each of 10 male Cx. tritaeniorhynchus, or 0.03 ml intracerebrally into each of 10 newborn mice, both equally susceptible to WN viral infection. Mosquitoes were held for 12 days at 28°C and then stored at −70°C for viral antigen assay. Mice were observed daily for 10 days for morbidity and mortality. Smears of mosquito abdomens and of mouse brain tissue from sick and dead mice were prepared on microscope slides. The smears were examined for WN viral antigen by the direct fluorescence antibody technique using a fluorescein isothiocyanate–conjugated WN virus hyperimmune ascitic fluid and a Leitz (Wetzlar, Germany) transmission fluorescent microscope at 400× magnification. Calculation of the minimum filial infection rates (MFIR) for pooled mosquitoes was based on one infected mosquito per pool.

RESULTS

A total of 120–200 females each of Cx. tritaeniorhynchus, Ae. albopictus, and Ae. aegypti was infected by inoculation with WN virus in an attempt to demonstrate vertical transmission of the virus. West Nile virus was recovered from F₁ adult progeny of each species (Table 1). The MFIR for adults ranged from 1:62 to 1:172 for Ae. aegypti, from 1:124 to 1:138 for Ae. albopictus, and from 1:325 to 1:859 for Cx. tritaeniorhynchus. The rate for Cx. tritaeniorhynchus was significantly lower ($P < 0.05$) than for the two Aedes species, which did not differ significantly from one another. However, the MFIR for the Ae. aegypti F₁ progeny in experiment 2 was significantly higher ($P = 0.01$) than the rate for progeny in experiment 1. An increase in the MFIR was also demonstrated for Cx. tritaeniorhynchus progeny in the second experiment, but the difference was not significant. Vertical transmission of WN virus by infected Cx. tritaeniorhynchus was also supported by the recovery of virus from larvae and pupae (Table 2). The MFIR

TABLE 1

Vertical transmission of West Nile virus by Aedes albopictus, Aedes aegypti, and Culex tritaeniorhynchus to F₁ adults

Experiment infection no.	Mosquito species	No. of F₁ tested	Pools		Minimal filial infection rate
			No. positive/ no. tested		
1*	Ae. albopictus				
	Males	371	3/8		1:124
	Females	275	2/6		1:138
1	Ae. aegypti				
	Males	2,243	13/45		1:172
	Females	1,239	10/26		1:124
2†	Ae. aegypti				
	Males	1,432	20/115		1:72
	Females	871	14/89		1:62
1	Cx. tritaeniorhynchus				
	Males	850	1/17		1:850
	Females	735	2/17		1:368
2	Cx. tritaeniorhynchus				
	Males	1,680	4/150		1:420
	Females	2,685	8/180		1:325

* All parent females in experiment no. 1 were infected with stock virus that had received 16 intracerebral passages in mice and four intrathoracic passages in Cx. tritaeniorhynchus

† Parent females in experiment no. 2 were infected with stock virus prepared from suspensions of infected parent mosquitoes from experiment no. 1.

was approximately the same for larvae maintained at 20°C and 26°C. The rate for pupae at each temperature was lower than that observed for larvae, with the rate being significantly lower ($P = 0.05$) for pupae maintained at 26°C.

The possibility that WN virus was transmitted vertically on the surface of eggs of infected mosquitoes was considered by maintaining uninfected first- and second-stage Cx. tritaeniorhynchus larvae in a suspension of $10^{7.3}$ suckling mouse intracerebral 50% lethal doses of virus for six hours. The adults that originated from these larvae were maintained at 28°C for 10–12 days, and then assayed for virus. Evidence of infection was not demonstrated in 1,600 of these mosquitoes, thus providing indirect evidence that WN virus was vertically transmitted inside the eggs.

DISCUSSION

Our results demonstrate that vertical transmission of WN occurs in mosquitoes. The virus was recovered from F₁ progeny of parenterally infected Ae. albopictus, Ae. aegypti, and Cx. tritaeniorhynchus. Although these data were generated by experiments involving parenterally infected mosquitoes, previous studies have demonstrated that other flaviviruses were vertically transmitted by mosquitoes irrespective

of the parenteral or oral route of infection.[23, 25, 27, 29–31] Experiments were not conducted to determine whether vertically infected females were capable of transmitting WN virus horizontally. Previous studies that demonstrate this route of transmission for other flaviviruses[22, 23, 26] support this as a likely possibility.

The filial infection rates for WN virus among F₁ adult mosquitoes indicated that the efficiency of vertical transmission for the two Aedes species was comparable, but was significantly higher than that observed for Cx. tritaeniorhynchus. These rates and those demonstrated for immature forms in this study were well within the range of rates reported for other flaviviruses ver-

TABLE 2

Vertical transmission of West Nile virus to F₁ immature progeny by parenterally infected Culex tritaeniorhynchus, according to the rearing temperature of immature forms

Progeny	Temperature (°C)	No. tested	Pools		Minimal filial infection rate
			No. positive/ no. tested		
Larvae	20	1,709	8/20		1:213
Pupae	20	1,563	4/18		1:390
Larvae	26	2,497	12/31		1:208
Pupae	26	1,661	3/21		1:554

tically transmitted by mosquitoes.[25, 27, 29, 30, 32-35] Also, our data demonstrating interspecific variation in transmission efficiency among mosquitoes is consistent with observations previously published for several other flaviviruses.[25-27, 29, 30, 32, 34, 35] While these experimentally derived observations have conclusively demonstrated that WN and several other flaviviruses can be vertically transmitted by mosquitoes, the role, if any, of this transmission route in nature has not been clearly defined for any of these viruses.

The efficiency of vertical transmission of WN virus by mosquitoes differed according to the species, as well as the passage level of the virus. Infection rates among *Ae. aegypti* and *Ae. albopictus* progeny were approximately the same when the parent females were infected with the lower-passaged stock virus that had received 16 passages in mice and four passages in mosquitoes. However, an increase was demonstrated among *Ae. aegypti* progeny of parent females infected with virus that had received an additional passage in *Ae. aegypti*. In addition, parent *Cx. tritaeniorhynchus* females infected with virus that had received an additional passage in females of this species transmitted virus to a slightly higher percentage of their progeny. Our data show that, at times, the rate of vertical transmission was increased if the infecting virus had been sequentially passaged in the mosquito homologous with that used for the experiment.

Only a few experiments have been conducted to determine the effect that the passage history of other flaviviruses may have on their vertical transmission by mosquitoes. Experiments involving Kunjin virus failed to demonstrate an effect of serial vertical passages on the filial infection rates for *Ae. albopictus*.[25] As part of the same experiment, a lower nonmouse-passaged strain of dengue 1 virus appeared to be transmitted vertically to a higher percentage of *Ae. albopictus* than an extremely higher mouse-passaged level of the same virus. Also, other investigators reported that the passage level of the virus appeared to affect vertical transmission of dengue and St. Louis encephalitis (SLE) viruses in *Ae. albopictus*.[26, 34]

Comparable filial infection rates were demonstrated among WN virus vertically infected *Cx. tritaeniorhynchus* larvae reared at 20°C and/or 26°C. Although no other relevant data have been reported for WN virus, these data were in agreement with SLE virus infection rates in larval progeny of *Aedes epactius*,[34] five *Culex* and two *Anopheles* species.[29] and dengue 1 virus in *Ae. cooki*.[35] In contrast, the filial infection rate for SLE virus in *Cx. pipiens* and *Cx. tarsalis*, especially *Cx. tarsalis*, was markedly higher for larvae reared at 18°C than at 27°C.[31] Another study showed a similar but less pronounced effect of temperature on SLE viral infection rates among *Ae. taeniarhynchus* larvae reared at 18°C and 27°C.[29]

While the SLE viral infection rates appeared to be enhanced among *Culex* species larvae reared at the lower temperature, the rate of transstadial transmission to adults was extremely low or nil regardless of the larval rearing temperature.[31] However, the higher SLE viral infection rate reported for *Ae. taeniarhynchus* larvae reared at 18°C as compared with 27°C was also observed among the adults derived from larvae reared at the lower temperature.[29] The lower rate observed among the larvae of this species reared at 27°C was reduced even further among adults reared from larvae at this temperature.[29] Also, the infection rate for Japanese encephalitis (JE) virus vertically infected *Cx. tritaeniorhynchus* larvae decreased during development to adults at temperatures ranging from 25°C to 30°C.[30] Similarly, our data suggested a decreasing pattern of WN viral filial infection rates among *Cx. tritaeniorhynchus*, but the change did not appear to be related to the larval rearing temperature. Finally, studies involving Kunjin, another flavivirus, and *Ae. albopictus* failed to demonstrate any effect of the temperature of rearing of immature forms on filial infection rates.[25] Although these observations indicated that the larval rearing temperature may affect vertical transmission of some flaviviruses, the data also revealed that another undescribed mechanism(s) led to a decrease in the transstadial transmission of these viruses.

Among the mosquito species investigated in this study, only *Cx. tritaeniorhynchus* has been implicated as a vector of WN virus.[1, 7, 8, 13, 17-20] However, our results were consistent with those of previous laboratory experiments that demonstrated vertical transmission of several flaviviruses in their respective incriminated vector species, as well as in species that were not recognized as vectors.[25, 29, 30, 31, 35] Whether WN virus is transmitted vertically by any of these mosquito species in nature is unknown. The virus was isolated once from *Ae. caballus* and from *Ae.*

circumlateolus during epidemics of WN fever in the Republic of South Africa.[4] The single isolations and the absence of an association of WN virus with other *Aedes* species suggest that vertical transmission might be uncommon among *Aedes* mosquitoes in nature.

Our demonstration of vertical transmission and the previous report show that *Cx. tritaeniorhynchus* can be infected with and transmit WN virus,[13] and the numerous reported isolates from India and Pakistan of WN virus support the possibility of both a vector and reservoir role for this species in nature. Additional support for the possibility that vertical transmission of WN virus occurs in nature can be found in studies demonstrating that such transmission occurs in dengue,[36,37] JE,[38] and yellow fever[39] viruses among naturally infected mosquitoes.

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